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(54) Title: AUTOCRINE MOTILITY FACTORS IN CANCER DIAGNOSIS AND MANAGEMENT

(57) Abstract

The present invention describes an isolated and substantially pure mammalian cell polypeptide which stimulates random locomotion of producer cell and which has a molecular weight greater than 30,000. The unique polypeptide of the present invention is inhibited by pertussis toxin. A kit and method for detecting metastasis in human are also described.

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AUTOCRINE MOTILITY FACTORS IN CANCER DIAGNOSIS AND MANAGEMENT

BACKGROUND OF THE INVENTION

Technical Field

The present invention is related generally to the field of cancer diagnosis and management. More particularly, the present invention is related to novel tumor motility factors and their utility in devising new approaches to cancer diagnosis, prevention and therapy.

State of the Art

Cell motility is necessary for tumor cells to traverse many stages in the complex cascade of invasion and metastases. Such stages include the detachment and subsequent infiltration of cells from the primary tumor into adjacent tissue, the migration of the cells through the vascular wall into the circulation (intravasation), and extravasation of the cells to a secondary site. The movement of cells through biological barriers such as the endothelial basement membrane of the vasculature may occur by means of chemotactic mechanisms. Studies on *in vitro* chemotaxis of some tumor cells indicate that a variety of compounds such as complement-derived materials, collagen peptides, formyl peptides, and certain connective tissue components can act as

chemoattractants. Todaro, et al. (Proc. Natl. Acad. Sci USA, 77:5258-5262, 1980) reported autocrine growth factors for transformed cells. Other growth factors of various types are also known. However, the existence and role of an autocrine factor controlling chemotactic (directional) and chemokinetic (random) motility of tumor cells has not heretofore been known or described. It may be important to note here that cell motility is an aspect of cell behavior distinct from cell growth and proliferation.

SUMMARY OF THE INVENTION

It is, therefore, an object of the present invention to identify and provide an autocrine factor controlling motility of tumor cells, such autocrine factor being designated herein as "AMF."

It is a further object of the present invention to provide antibodies having specific binding affinity for AMF or AMF receptors.

It is a still further object of the present invention to provide a kit for detecting, localizing and predicting metastases and tumor angiogenesis in humans.

It is yet another object of the present invention to provide a method of predicting, preventing and/or treating metastatic invasion and cancer proliferation in humans.

It is an additional object of the present invention to provide a pharmaceutical composition comprising an effective amount of neutralizing antibodies against AMF to inhibit motility of tumor cells in a pharmaceutically acceptable carrier.

Various other objects and advantages of the present invention will become evident from the Detailed Description of the Invention.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other objects, features and many of the attendant advantages of the invention will be better understood upon a reading of the following detailed description when considered in connection with the accompanying drawings wherein:

Fig. 1 shows a schematic representation of the Boyden test; and

Fig. 2 shows (a) Scatchard analysis of ^{125}I-AMF binding to suspended tumor cells; and (b) dose response curve of cell motility to purified AMF.

DETAILED DESCRIPTION OF THE INVENTION

The above and various other objects and advantages of the present invention are achieved by a polypeptide having the following properties: (a) secreted by mammalian cells and stimulates random locomotion of the producer cells; (b) having molecular weight of > 30,000; and (c) being inhibited by pertussis toxin. The polypeptide of the present invention is found to have, at least in part or in whole, the following amino acid sequence at its NH_2 terminus (single letter code) or at the NH_2 terminus of an active fragment of the polypeptide:

D K E L R F R D C T K S L A E A N K K

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

MATERIALS AND METHODS

Cell Lines

Human MDA231 and MDA435 breast carcinoma cells lines were obtained from ATCC and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. Both of these estrogen independent cell lines produce metastases in the lungs of a 6 week-old NIH nude mice, 6 weeks following injection of 5×10^5 cells into the lateral tail vein.

Isolation and Purification of the Autocrine Motility Factor

MDA231 and MDA435 human breast carcinoma cells are grown in DMEM to 60% confluency in the absence of added protein. The media is lyophilized and the residue dissolved in about 2 ml of distilled H_2O. This solution is applied to a PD-10 (Sephadex G25 medium) column. The first 2.5 ml are discarded and the next 4 ml are collected. The effluent contains AMF separated from low molecular weight material. This collected fraction is made up to 0.02 M phosphate buffered saline, pH 7.4 (PBS) with 10 x PBS and applied to a Sephacryl S-300 column in PBS (source of column). Elution with PBS

yields an active fraction that corresponds to material with a molecular weightof about 54 kDa. This fraction is dialyzed and concentrated 25 fold. The material is made up to 50 mM Tris-acetate, pH 8.0 and applied to a mono Q anion exchange column (source) and eluted with a linear salt gradient (0-1 M NaCl) with the following modification: When the NaCl concentration reaches 0.25 M, this concentration is held for 10 min before resuming the gradient. AMF is eluted in the 0.3 M to 0.4 M NaCl fraction. The active fraction is dialyzed and concentrated to a small volume (about 0.5 ml) which in turn is made up to 0.02 M phosphate in normal saline, pH 7.4. This is applied to a heparin column in PBS. The column is eluted with a linear gradient of NaCl (0.15 M to 1 M) which elutes AMF between 0.35 M and 0.4 salt gradient. After each purification step, column fractions (dialyzed to remove salt) are assayed for motility stimulating activity by the modified Boyden chamber procedure.

Assay Procedure for Cell Motility

The assay of motility is accomplished by the use of a modified Boyden (Zigmond, et al, J. Exp. Med. 137:387-410, 1973) chamber. This is a device (Figure 1) consisting of 2 wells horizontally separated by a microporous polycarbonate filter with a pore diameter of about 8 u. The motility stimulus (or chemoattractant) is placed in the lower well to contact the filter. To the upper well is added a suspension of cells (for example A2085 melanoma cells) at a concentration of about 10^6 cells/ml. The chamber is then placed in a humidified incubator for about 4 hours at 37 degrees C in an atmosphere of air and about 5% CO_2. During this time, the cells are deposited by gravity on the topside

of the filter. However, some cells (about 5 to 10%) migrate to the underside of the filter in response to the motility stimulant. Expenditure of energy must occur during migration since the average diameter of the cell is greater than the pore size diameter. At the end of the incubation period, the filter is removed and subjected to a fixing and staining procedure. This includes first immersing the filters in a methanol-containing solution for about 2 minutes; then in an eosin solution for about 2 minutes; and then in a hematoxylin solution for about 3 minutes. Thereafter the filters are washed in water and placed on a glass slide with the topside up. The buttons of stained cells on the topside are completely removed with a small piece of dry tissue paper. The stained cells that have migrated through the filter then become apparent. These are counted with the aid of a microscope at a magnification of about 500X. Five different high power fields are visualized with a grid in one ocular, the cells in 5 fields are counted and the average is computed. A ratio of $\geq$5 for positive control/negative control is indicative of a positive response of the cells to the motility stimulus.

Determination of Random and Directed (Chemotactic) Motility

Measurement of random motility is accomplished by exposing the cells to a fixed concentration of stimulus and determining their migration as described above. This includes adding equal increasing concentrations of attractant to both upper and lower wells prior to the assay incubation. The random migration of cells as a function of the levels of attractant is then determined. Directed migration occurs if the cells migrate better in

positive gradients (higher concentrations of attractant in the lower well compared to the upper well) than in negative gradients (higher concentrations in upper well than in lower well). The results of such an assay are shown in the "checkerboard" tabulation of the results (Table 1). It can be seen that random motility is quite significant for the A2058 melanoma cells responding to the AMF.

TABLE 1

% Motility Factor in Upper Well

% Motility Factor In Lower Well	0	15	30	45
0	100	244	512	494
15	494	1056	825	1469
30	1781	1550	2144	2640
45	2800	2550	2262	4362

'diagonal' shows random migration of cells. Lower triangle shows directed migration of cells in a positive gradient of motility stimulus.

Assay for Cell Pathways Involved in AMF Induced Motility

Materials: DMEM supplements with L-glutamine (2 µg), penicillin and streptomycin with or without 10% heat-inactivated fetal calf serum were purchased from commercial sources such as Meloy Laboratories, Inc. (Springfield, VA). Pertussis toxin and cholera toxin were obtained from List Biological Laboratories, Inc. (Cambell, CA). Phorbol 12-myristate 13-acetate (PMA), phorbol 12, 13-didecanoate (PDD), calcium ionophore A23187, diltiazem, nifedipine, verapamil, trifluoperazine, leupeptin, forskolin and 8-Br cAMP were all purchased from Sigma Chemical Company (St. Louis, MO). The 1-oleoyl-2-acetylglycerol was from Molecular Probes (Eugene, OR). The Nucleopore membranes (polyvinyl- pyrrolidone-free) as well as the 48-well chemotaxis chamber were purchased from Neuro Probe, Inc. (Cabin John, MD).

Cell Culture: The human melanoma cell line A2058 was maintained as described by Todaro et al, supra.

Production of Autocrine Motility Factor: In a modification of the previously described technique (Liotta et al, Proc. Natl. Acad. Sci. USA 83:3302-3306, 1986), A2058 cells were innoculated for 48 hours in DMEM without any protein supplement. The medium was concentrated using a Centricon ultrafiltration assembly, molecular weight cut off 30,000 daltons.

Chemotaxis Assay: The assay used to determine cell motility was a modification of the techniques described by Harvath et al, 1980... Liotta et al, 1986 supra. In accordance with this technique A2058 melanoma cells (approximately 75-90% confluent) were harvested with trypsin-EDTA and allowed to recover at room temperature in DMEM supplemented with 10% fetal calf serum for at least one hour. The cells were then resuspended at

2 x 10^6/ml in DMEM with 1 mg/ml bovine serum albumin. The assay was performed in 48-well micro-chemotaxis chamber (Harvath et al, 1980 supra) with 8 µm Nucleopore membranes coated with type IV collagen. The chambers were incubated at 37 degrees C for 4-5 hours, then developed using Diff Quick stains (American Scientific). The stained membranes were placed onto glass slides with the original cell side up so that the cell pellet could be wiped from the surface. Cells that had migrated through the pores were trapped between glass and membrane and could be easily counted by light microscopy under high power field (500X). Unstimulated random migration was <20% of directed migration.

Prior to or during the chemotaxis assay, chemicals could be co-incubated with cells to alter cellular metabolism or stimulate a chemokinetic response. At the start of the assay, chemicals could also be added to the lower chamber to demonstrate chemotactic potential.

Production of Murine Antibodies to AMF

Purified AMF protein (10 µg) was emulsified with complete Freund's adjuvant and injected into the foot pad of 3 C3H mice. Two weeks later the mice were boosted with 5 ug of AMF in PBS injected intravenously in the tail vein in a volume of 0.1 ml. One month later the mice were bled and the serum was tested for its ability to inhibit tumor cell motility. In this assay the mouse sera was preincubated with the AMF in the Boyden chamber migration assay. At a dilution of 1/1000 the mouse sera produced 90% inhibition of tumor cell motility compared to pooled mouse sera control. Purified AMF protein (10 µg) was emulsified in complete Freundi's adjuvant and injected into a subcutaneous site on the back of New Zealand white rabbits. Booster injections of 5 µg were

applied at 6 and 12 weeks. At 3 and 4 months the rabbits were bled and the sera was tested for motility inhibition activity. At a dose of 1/1000 the immune sera abolished motility compared to control preimmune sera. The sera were heat inactivated at 56°C for 30 minutes.

Determination of AMF Purity

The purity of the isolated AMF was determined by the following criteria:

(a) Single 54 kDA band was found on a single and two dimensional polyacrylamide gel electrophoresis performed by standard procedures well known in the art. Protein was identified with silver stain.

(b) Protein band cut from the gel retains motility stimulating activity.

(c) NH_2 terminus amino acid sequence (1-19) reveals one type of amino acid residue at each cycle; and

(d) Murine and rabbit anti-AMF antibodies block the motility stimulating activity of human tumor AMF.

Based on the above criteria, the isolated AMF of the present invention was found to be substantially pure. The term "substantially" as used herein means as pure as it is possible to obtain by standard techniques.

Amino Acid Sequencing

Edman degradation of purified AMF is performed with the Applied Biosystems (Foster City, CA) model 470A gas-phase sequencer using the trifluoracetic acid chemistry provided by the manufacturer. The phenylthiohydantoin amino acids were identified and quantitated by using the Perking-Elmer series 3B HPLC and ultraviolet detection.

Dose Response and Time Course of Pertussis Toxin and Effect on Motility: Pertussis toxin (PT) was added to A2058 for overnight culture in flasks, for various period of preincubation prior to an assay, or at different times after the start of an assay. PT doses that were tested ranged from about 10 ng/ml to 1.5 µg/ml. Cell viability at any of the tested doses was comparable to the viability in untreated control (>90%). Treated and untreated cells were then tested for their motility response to the A2058 conditioned medium. Cell motility in response to the DMEM alone was included as a negative control for each treatment group of cells.

Overnight incubation of the cells with any of the tested PT doses resulted in significant inhibition of cell motility (Table 2). Preincubation for 30 minutes to 2 hours at doses of 0.5 - 1.5 µg/ml also resulted in greater than 50% inhibition. When pertussis toxin was added at the start of the assay or later, there was a gradual diminution in the inhibitory effect. By 1-2 hours after the start of the assay, PT had minimal effect on the observed motility.

The dose response of PT was consistent with previously described inhibitory doses of PT for G_i and G_o proteins. The time course showed much diminished inhibition when PT was added at inadequate doses or for insufficient time to saturate the G protein sites. Hence, the data obtained in the present testing was consistent with the hypothesis that AMF stimulates cell motility through a receptor which requires a G protein to activate the cells.

TABLE 2

AMF TREATMENT DATA

A.	Treatment	Motility (% of Controls)
1	P'ase K	13.2
2	DNAase 2 g/ml	95.1
3	RNAase	104
4	PMSF 5 mM	95.5
5	DDT 10 mM	11.5
6	Heating 100C	5.0
7	Heating 56C	97.2
8	pH 4.0	20
9	pH 7.4	100
10	pH 11.0	100

PERTUSSIS TOXIN INHIBITION OF AMF INDUCED MOTILITY

B.	Time Pertussis Toxin Added (hrs. from start of assay)	Percent Inhibition of AMF Induced Motility
	-2.0	100
	-1.0	95
	-0.5	100
Start of Assay*	0	62
	+0.5	55
	+1.0	33
	+2.0	0 (no inhibition)*+
	+3.0	0 (no inhibition)*+

*Time of addition of AMF

*+Pertussin Toxin requires at least 1 hour to penetrate cell membranes and inhibit G proteins by ADP ribosylation.

Cholera Toxin Dose Response and Time Course: Cholera toxin (CT) in contrast to pertussis toxin, is thought to act on the G_S protein that stimualtes adenylate cyclase to produce the second messenger, cAMP. Cholera toxin was added to A2058 cells either for overnight incubation in flasks or for variable periods of preincubation prior to the start of the chemotaxis assay. The tested doses of cholera toxin ranged from about 0.1-50 µg/ml. At all tested doses, cell viability was comparable to that of untreated cells (>90%). Treated and untreated cells were then tested for chemotactic response to A2058 conditioned medium.

Overnight treatment with CT caused a diminished response to the A2058 conditioned medium, though the inhibitory effect was never complete (30-60% inhibition). If the cells were exposed to cholera toxin for just a brief preincubation prior to the start of the chemotaxis assay, the inhibition was minimal (<5%).

Effect of Other Agents Involved in the Adenylate cyclase System on Cell Motility: Cholera toxin is thought to act by ADP-ribosylation of the G_S protein in an active configuration that can stimulate adenylate cyclase. Since the effect of cholera toxin on A2058 cell motility was minimal, further tests were conducted to determine whether other agents that act on the cAMP pathyway would be inhibitory. Forskolin stimulates adenylate cyclase directly without acting through an intermediary G protein. The cAMP analogue, 8-Br cAMP, is able to enter intact cells. Both chemicals were added to A2058 cells either for overnight incubation in flasks or for a 2 hour preincubation prior to the start of chemotaxis. Both exhibited only a partial inhibition of cell motility that

was essentially identical to that of cholera toxin for comparable periods of time.

Since these cells respond in a dose-dependent manner to various concentration of conditioned medium obtained by incubating confluent cells in serum-free medium, it was concluded that the motility factor is derived from the cell. Results obtained with the modified Boyden chamber experiments also demonstrate that the autocrine factor of the present invention has both chemotactic (directional) and chemokinetic (randomly motile) properties. Since the random stimulation was found to be about three-fold greater than the directed motility, it was concluded that the cells respond to gradients of the motility factor as well as to high uniform concentrations of the attractant.

When determined by gel filtration and gel electrophoresis, the migration-stimulating material of the present invention is found to have a molecular weight of about 54 kilodaltons. This form may be a precursor of an active factor. It is possible that cellular or serum components could activate or inhibit the action of the motility factor. The motility factor is inactivated by exposure to streptococcal protease, but active chymotrypsin-derived fragments can be produced (data not shown). The activity is destroyed by boiling but is stable upon exposure to 56 degrees C. Additionally, the activity is stable to a pH range from 4 to 11 (data not shown). These properties indicate that the autocrine material (AMF) of the present invention is different from a variety of known growth factors and chemoattractants. It was also found that known growth factors such as PDGF, αTGF, βTGF, EGF, IGF, transferrin, or FGF do not substitute or block the AMF (data not shown). Amino acid analysis indicated a unique sequence of 19 amino terminal

amino acids of AMF. A slightly small form of the active material was also found to have a unique amino terminal sequence. Protein data base searches failed to reveal any other polypeptide with such a sequence.

It has also been found that motility induction by AMF is not blocked or substituted by known growth factors or serum factors. At a concentration of 1 nM or less, AMF markedly stimulates the random and directed motility of breast cancer cells but fails to induce motility in leukocytes. The factor also stimulated random pseudopodia production by breast carcinoma cells and melanoma cells. Following trasfection with the activated ras-oncogene, AMF and its receptor are enhanced more than 100 fold in certain cells. Human breast carcinoma cells, but not normal breast epithelium, produce large quantities of AMF. Antibodies recognizing AMF abolish human tumor cell motility in vitro without altering tumor cell viability.

The availability of an isolated and purified autocrine, polypeptide, tumor motility factor makes it possible to obtain anti-AMF antibodies having specific binding affinity for said motility factor. Such antibodies can either be polyclonal or monoclonal and are prepared by well known standard techniques routine in the art. Such antibodies can also be labelled with suitable radioisotopes or fluorescent and other markers or ligands and employed for the detection, quantitation and/or localization of the AMF in human tissue or body fluid. Furthermore, radiolabelled AMF together with unlabelled AMF can be utilized in a standard competitive assay to measure AMF receptor level. Such binding assay for determining the receptor level is carried out as follows.

AMF Binding Assay:

Purified AMF is iodinated using the standard Bio Rad enzymobead procedure. Increasing amounts of labeled AMF is incubated in a volume of 1 ml with 100,000 A2058 melanoma cells, in the presence or absence of 100 fold excess cold competitor. Incubation is conducted at 37°C for 40 minutes and the cell-bound radioactivity is separated by centrifugation. AMF binding exhibits saturation with 80% specific binding and about 30,000 receptors per cell. Scatchard analysis according to standard methods shows a linear relationship between the specifically bound/free ratio and the specifically bound AMF, with an estimated kd in the range of about 0.5 nM.

Detection of cancer in humans is also made possible by the present discovery and testing of human body samples for this purpose is now illustrated using urine samples from bladder cancer patients.

Urine samples from patients with bladder cancer are collected and processed with centrifugal microconcentrator (AMICON) with an exclusion filter of 10 kilodaltons. The processed urines are reconstituted at a 10-fold concentration with steriel phosphate buffered saline pH7.5 and stored at -20°C until use. Tumor grade is determined by a pathologist using a scale of one to three with grade one tumors showing the most differentiation and grade three tumors showing the least differentiation. Bladder tumors are staged according to the American Joint Committee TNM classification.

Assay of Urine Samples:

Although any cell line which responds to AMF can be employed, the preferred cell line is human MDA 435 cells (ATCC). The concentrated urine samples are applied to the microwell migration chamber assay as described herein

supra. Each sample is tested at a series of dilutions with and without the addition of the antibodies directed against human tumor AMF. AMF units are recorded as the proportion of tumor cells stimulated to migrate by the sample which is inhibited by the antibodies. In general, greater than 80% of the stimulated migration is inhibited by an antibody concentration of about 10 µg/ml.

As shown in Table 3, control urines with non-neoplastic disorders such as kidney stones failed to contain significant levels of motility factors. All of the bladder transitional cell carcinoma cases exhibited a positive motility response in the urine. The highest levels of motility factor production was found in the urine of patients with high grade tumors or with stage D (metastatic) tumors.

TABLE 3

	Urine Sample	AMF units	SE
1	Control ks 75	5	.5
2	Control ks 76	9	2
3	Ca in Situ	32	5
4	Papillary TCC	64	8
5	TCC 77	44	3
6	TCC 69	98	14
7	TCC 73	123	32
8	Recur TCC 79	130	22
9	TCC II 485	169	14
10	TCC II 491	105	8
11	TCC II 554	41	12
12	TCC III 457	72	6
13	TCC stg D 584	234	25

TCC = Transitional cell carcinoma of the bladder
Recur TCC = Recurrent TCC
TCC II = grade II
TCC III = grade III
TCC stg D = metastatic TCC
KS = Kidney stones
SE = Standard error

Of course, the antibodies against AMF can be employed to block or inhibit AMF activity thereby arresting tumor invasion or metastatic proliferation which depend on tumor cell motility. Availability of such neutralizing antibodies also makes it possible to treat such conditions as breast carcinoma and melanoma by administering to a person inflicted with these conditions, an effective amount of the AMF-antibodies to prevent these conditions from progressing. A pharmaceutical composition for treating cancer and metastases is prepared by simply including an effective amount of neutralizing antibodies against AMF to inhibit motility of tumor cells and a pharmaceutically acceptable carrier such as physiological saline, non-toxic buffers and the like.

Means for detecting tumor aggressiveness and/or metastatic activity is now also made possible by a kit comprising separate containers containing (a) antibodies having specific binding affinity for AMF; (b) labelled AMF; (c) unlabelled AMF and instructional material for performing tests utilizing the antibodies and the AMF provided in the kit for determining AMF and/or receptor activity in a body sample. Such accessories as microtiter plates, micropipettes, means for reading antibody titer and the like are routinely found in such kits and may be included for convenience in the kits of the present invention.

In summary, the present invention provides a new tool for understanding mechanisms which control tumor cell invasion and opens new strategies for cancer diagnosis and therapy. Epithelial cells do not normally exhibit invasive behavior. The motility factor described herein does not affect the migration of normal blood leukocytes. Therefore, a therapeutic agent aimed at

inhibiting the factor described in the invention should have low toxicity against normal resting tissues. Pharmacologic preparations obtained in accordance with the present invention which inhibit invasion of tumor cells and prevent the transition from in situ to invasive carcinoma could be potent cancer arresting agents. Inhibitors of tumor invasion can also prevent the growth of established metastases because a metastasis may need to invade locally as it grows. Furthermore, such agents may inhibit tumor angiogenesis. Antibodies to motility factors or their receptors could be applied through tissue immunohistology, radioscintography, or serum immunoassays to localize metastases and predict cancer aggresseiveness in individual patients. As gene products, autocrine motility factors or their receptors define a new class of oncogenes. The level of expression of these genes in a patient's tumor may provide important diagnostic information through monitoring the level of AMF in the body sample.

Of course, invasion and metastases are among the major causes of cancer treatment failure. The present invention provides new clinical strategies to (a) detect pre-invasive lesions and prevent their progression; (b) accurately predict the aggressiveness of a patient's tumor, and (c) identify and eradicate micrometastases. One of the least understood aspects of tumor invasion is tumor cell locomotion. The present invention allows the determination of the role of the tumor cell motility factor.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated and substantially pure mammalian cell polypeptide which stimulates random locomotion of producer cell, having a molecular weight >30,000 and being inhibited by pertussis toxin.

2. The polypeptide of claim 1 having at NH_2 terminus, an amino acid sequence, at least in part, as follows:

D K E L R F R D C T K S L A E A N K K.

3. Antibodies having specific binding affinity for the polypeptide of claim 1.

4. A method for arresting metastatic proliferation comprising administering to a host suspected of or inflicted with malignant tumors an effective amount of antibodies of claim 3 to inhibit tumor proliferation.

5. The method of claim 4 wherein said malignant tumors are melanoma, breast and bladder carcinoma.

6. A kit for detecting tumorogenic or metastatic activity in a body, comprising a container containing antibodies having specific binding affinity for autocrine motility factor (AMF).

7. A kit for determining the level of AMF cell receptors comprising containers separately containing (a) labelled AMF; (b) unlabelled AMF; and (c) instructions for performing tests with a body sample to determine the level of AMF-receptor activity.

8. A method for detecting the presence of carcinoma in humans comprising reacting human body sample from a patient suspected of having carcinoma with a cell line susceptible to AMF and determining motility induced in the susceptible cell line by said body fluid.

9. The method of claim 8 wherein the motility incuded by said human body sample is inhibited by anti-AMF antibody.

10. The method of claim 8 wherein said carcinoma is human bladder, breast or lung carcinoma.

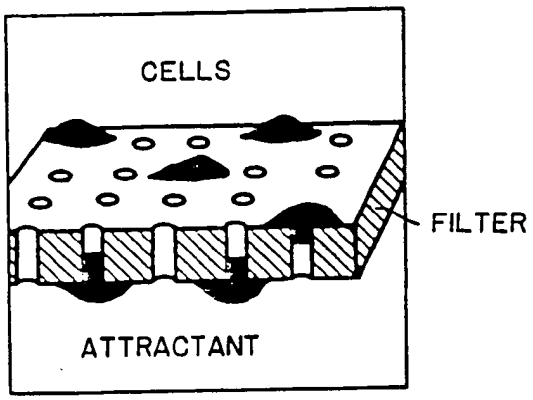


FIG. 1

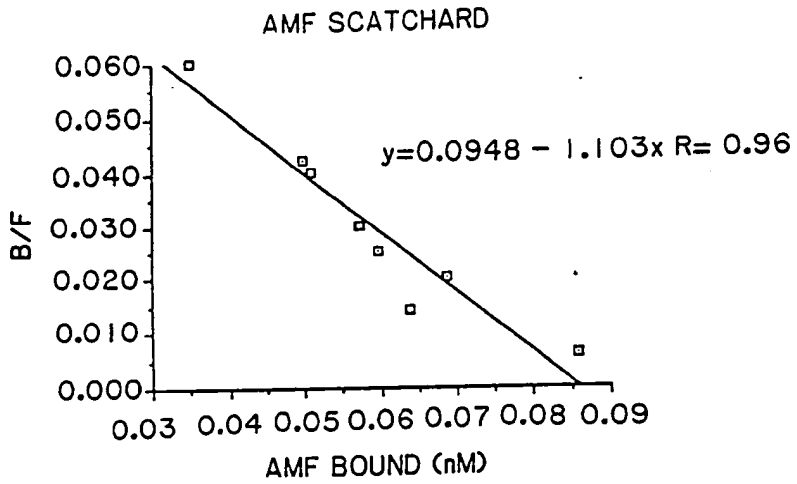


FIG. 2(a)

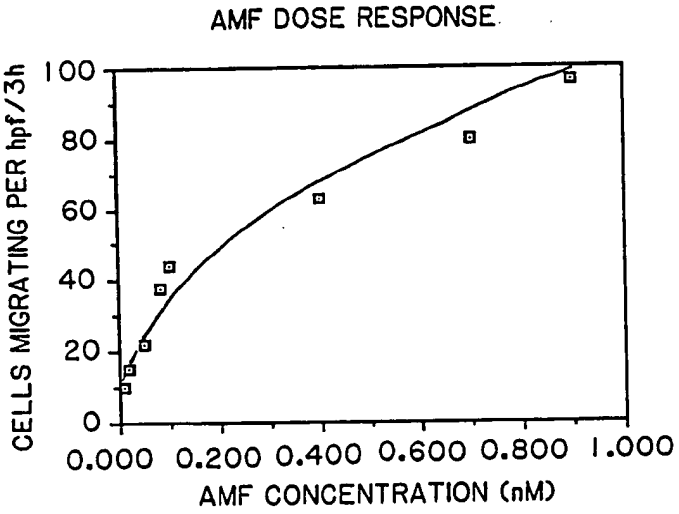
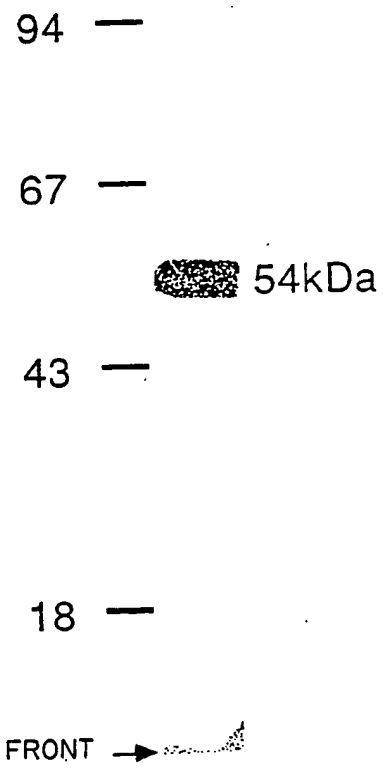


FIG. 2(b)



SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

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I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

U.S. CL. 530/324,350,387;514/21;436/547,503;435/29
INT. CL. (4): C07K 15/00,7/10;A61K 37/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
US	530/324,350,387;514/21;436/547,503;435/29

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Chemical Abstract, Vol. 105, Issued July 1986 (Columbus,OH.)abstract no. 22367p(Liotta) "Tumor Autocrine motility factor"	1-10
A,P	Chemical Abstract, Vol. 108, Issued January 1988,(Columbus, OH.) abstract no. 19880g (Guirguis)"Cytokine-induced pseudopodial protrusion is couple to tumor cell migration"	1-10
A,P	Chemical Abstract, Vol. 108, Issued February 1988, (Columbus, OH.)abstract no. 53562q. "Biochemical mechanisms of tumor invasion and metastasis"(Liotta)	1-10

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 JULY 1988	01 SEP 1988
International Searching Authority	**Signature of Authorized Officer**
ISA/US	DELBERT R. PHILLIPS

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No **
X,P	Biochemical and Biophysical Research Communications, Vol. 146, No.1,Issued July 1987, pages 339-345,(Stracke) "Pertussis Toxin-Inhibits Stimulated Motility Independently of the Adenylate Cyclase Pathway In Human Melanoma Cells" (Bethesda, MD.)See summary.	1-10
X	Proceeding National Academy of Science, Vol. 83, pages 3302-3306, Issued May 1986, (Liotta)"Tumor cell autocrine motility Factor" See abstract .(Washington,D.C.)	1-10